ARTICLES

Prevalence of antibodies to infectious bovine rhinotracheitis, parainfluenza 3, bovine respiratory syncytial, and bovine viral diarrhea viruses in cattle in Saskatchewan and Alberta

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Abstract

A total of 1745 healthy cattle from 295 farms in Saskatchewan and Alberta was tested by ELISA for antibodies to four viruses. Antibodies to infectious bovine rhinotracheitis (IBR) virus were found in 37.8% of sera (59.5% of properties), to parainfluenza 3 (PI3) virus in 93.9% of sera (99.7% of properties), to bovine respiratory syncytial (BRS) virus in 78.5% of sera (86.6% of properties), and to bovine viral diarrhea (BVD) virus in 40.6% of sera (66.7% of properties).

The prevalence of PI3 viral antibodies among Saskatchewan cattle was not affected by district of origin, breed, sex, age, or vaccination practices, though BRS viral antibodies appeared less frequent in young, male, and unvaccinated animals. Antibodies to IBR and BVD viruses were less prevalent in the Prince Albert/Tisdale districts and in young, male, and unvaccinated animals, but were more common in Holstein cattle. Antibodies to IBR virus appeared less frequent in Herefords. Antibodies were more prevalent in cattle which had been vaccinated against IBR, BRS, and BVD virus infections.

The relatively small number of cattle sampled from Alberta had a similar prevalence of antibodies to PI3 and BRS viruses to that seen in cattle in Saskatchewan, though IBR and BVD prevalence rates were lower.

Résumé

Prévalence des anticorps contre la rhinotrachéite infectieuse bovine, parainfluenza 3, le virus respiratoire syncytial bovin et la diarrhée à virus bovine chez des bovins de la Saskatchewan et de l'Alberta

Des tests ELISA détectant la présence d'anticorps contre quatre virus différents furent effectués sur le sérum de 1745 bovins en bonne santé provenant de la Saskatchewan et de l'Alberta. Des anticorps contre le virus de la rhinotrachéite infectieuse bovine (IBR) furent retrouvés dans 37,8 % des sérums (59,5 % des fermes), contre le virus du parainfluenza 3 (PI3) dans 93,9 % des sérums (59,5 % des fermes), contre le virus respiratoire syncytial bovin (BRS) dans 78,5 % des sérums (86,6 % des fermes) et contre les virus de la diarrhée bovine (BVD) dans 40,6 % des sérums (86,7 % des fermes).

Quoique les anticorps contre les virus BRS apparurent moins fréquents chez de jeunes animaux mâles et n'ayant pas été vaccinés, la prévalence d'anticorps contre le virus PI3 chez les bovins de la Saskatchewan ne fut pas affectée par le district d'origine, la race, le sexe, l'âge ou la pratique de la vaccination. La prévalence des anticorps contre les virus IBR et BVD fut moindre dans les districts de Prince Albert/Tisdale et chez de jeunes mâles qui n'avaient pas été vaccinés. Par contre, la prévalence était plus élevée chez des bovins Holstein. Des anticorps contre le virus IBR furent retrouvés moins fréquemment chez les bovins de race Hereford. Les anticorps furent retrouvés plus fréquemment chez des bovins vaccinés contre les infections à virus IBR et BVD.

Le petit nombre d'échantillons prélevés chez des bovins de l'Alberta avaient, comparativement à ceux de la Saskatchewan, une prévalence similaire en ce qui concerne les anticorps contre PI3 et BRS quoique celle contre IBR et BVD était plus basse.

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Introduction

Acute respiratory disease continues to be a substantial problem in cattle in western Canada, and sometimes causes considerable illness in young cattle following their entry to feedlots. Parainfluenza 3 (PI3), bovine respiratory syncytial (BRS), and infectious bovine rhinotracheitis (IBR) viruses have all been incriminated in the etiology of acute respiratory disease in cattle in Canada (1-5). It has also been suggested that bovine viral diarrhea (BVD) virus may contribute to outbreaks of respiratory disease (4,6).

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There is little information available on the current distribution and prevalence of antibodies to these viruses in the cattle population of western Canada. Although several surveys of the prevalence of antibodies to these viruses have been reported (7–11), most were conducted many years ago when vaccination practices were markedly different from the present.

In this report we detail the results of a recent serological survey of cattle in Saskatchewan and Alberta for antibodies to the IBR, PI3, BRS, and BVD viruses.

Table 1. Prevalence of antibodies to IBR, PI3, BRS, and BVD viruses in cattle sera

		Prevalence of antibodies (%) to:			
Number tested		IBR	PI3	BRS	BVD
Total animals:	1745	37.8	93.9	78.5	40.6
Farms:	295	59.5	99.7	86.6	66.7

Materials and methods

Serological procedures

An enzyme-linked immunosorbent assay (ELISA) for antibodies to IBR virus was carried out as described by Durham and Sillars (12), except that 0.004 M orthophenylene diamine (Sigma, St Louis, Missouri, USA) was used as the chromogen.

Antigens for the PI3 and BRS ELISA were prepared by inoculating heavy doses of the viruses onto Vero cells maintained in Earle's minimal essential medium (Gibco, Grand Island, New York, USA) containing 5% fetal bovine serum (FBS) (Flow Laboratories, McLean, Virginia, USA), 1% nonessential amino acids (Gibco), 10% lactalbumin hydrolysate (Difco, Detroit, Michigan, USA) and antibiotics. Once cytopathic effect became visible, the medium was harvested every two days and replaced by fresh medium until the cells were destroyed. The harvested culture fluids were clarified by centrifugation at 600 *g* for 20 minutes, then stored at −80 °C. Any cells recovered from the medium were returned to the culture.

After destruction of the monolayer, residual cells and medium were frozen and thawed two times and sonicated at 20 kHz for 15 seconds using a 70% pulse mode with an ultrasonic processor (Sonics and Materials, Danbury, Connecticut, USA). The tissue suspension was centrifuged at 600 *g* for 20 minutes and the clarified fluids pooled with previously harvested, clarified media. The fluids were then ultracentrifuged at 100,000 *g* for three hours at 4°C, and the pellet resuspended to 10% of the original volume in phosphate-buffered saline and stored at −80°C. Uninfected cell cultures were processed similarly for use as cell control antigen. Optimal concentrations of antigen were established by titration against standard positive and negative control sera.

The PI3 and BRS ELISA procedures followed a protocol similar to that described for the IBR ELISA, except that 4% polyethylene glycol (MW 8000) (BDH Chemicals, Toronto, Ontario) was added to the conjugate diluent to intensify the reaction.

The BVD ELISA was carried out using a recently developed test procedure (13) utilizing peroxidase-labelled protein G.

All ELISA results were calibrated against their respective standard positive control sera and a negative control serum (FBS) to give uniformity to the results over the period of time that tests were performed. The optical density (OD) of the plates was read in a plate reader (Titertek Multiskan, Flow Laboratories) at 492 nm, and the results assessed via a coupled microcomputer. The net reactivity of the sera was assessed as the OD of wells with viral antigen minus the OD of wells with cell control antigen, all values being blanked against the net OD of the FBS. The final results were expressed in units as follows:

$$\frac{\text{Mean net blanked OD of test serum}}{\text{Mean net blanked OD of positive standard serum}} \times 100$$

Test results were classified as positive or negative on the basis of ELISA reactivity greater than ten units. This was equivalent to serum neutralization test titers of 1:3 for IBR, PI3, and BRS viruses, and 1:2 for BVD virus, based on comparative studies with 100 test sera with each of the viruses.

Control sera

Standard positive control sera for the IBR, PI3, and BRS tests were obtained from cattle with high neutralizing antibody titers against the respective viruses. The BVD standard positive control serum was prepared by vaccinating a cow with a killed BVD vaccine (Triangle 1, Fort Dodge Laboratories, Fort Dodge, Iowa, USA), followed by two intramuscular injections at weekly intervals of 10^6 $TCID_{50}$ of a local isolate of BVD virus. Antisera to the four viruses all had SNT titers of 1:256. Fetal bovine serum that was free of antibodies to the four viruses was used as negative control serum.

Test sera

These were obtained from a number of sources:

(a) Nine hundred and thirty sera were collected from healthy adult cattle. These sera were submitted for brucellosis certification purposes to the Health of Animals Laboratory, Saskatoon (Agriculture Canada) from various areas of Saskatchewan. Individual owners' names were not divulged to us.

(b) Two hundred and sixty-seven sera were collected from healthy bull calves entering the Record of Performance (ROP) bull test station in Saskatoon, Saskatchewan.

(c) Two hundred and fifty-three sera were obtained from locally reared calves at the Melfort Research Station, Saskatchewan.

(d) Fifty sera were collected from locally reared calves on entry to a commercial feedlot near Calgary, Alberta.

(e) Eighty sera were submitted from bull calves on entry to the Lakeland bull test station at Vermilion, Alberta.

(f) One hundred and fifty-three sera were submitted to this laboratory from healthy cattle in Saskatchewan.

Table 2. Prevalence of antibodies to IBR, PI3, BRS, and BVD viruses within Saskatchewan (by veterinary district) and in Alberta

Veterinary districts	Number of:		Prevalence of antibodies (%) to:			
	Sera	Properties	IBR	PI3	BRS	BVD
Lloydminster and North Battleford	121	37	52.8	95.0	81.8	55.3
Prince Albert and Tisdale	420	15	9.0	91.6	75.2	14.8
Saskatoon and Humboldt	294	68	45.9	95.9	71.0	47.6
Moose Jaw and Regina	288	46	52.1	90.3	82.3	37.1
Swift Current, Maple Creek and Assiniboia	175	32	47.4	86.9	70.3	52.6
Weyburn, Moosomin and Yorkton	318	65	64.1	98.7	90.9	67.3
Alberta	129	32	20.2	99.2	72.1	22.5

Serological survey

All sera used in the survey were tested by ELISA against the four viral antigens. Records were kept as to district of origin, age, breed, sex, and vaccination history of the cattle (where available). The test results were evaluated using chi-squared analysis.

Results

A total of 1745 sera from healthy cattle on 291 properties was tested during the survey. The overall results are shown in Table 1. The property data should be regarded as conservative, as sample sizes varied considerably, ranging between 1 and 99 with a mean of 5 (excluding the 253 sera received from the Melfort Research Station).

Cattle with antibodies to IBR virus showed a mean activity of 53.5 ± 33.7 units, equivalent to an IBR SNT titer of about 1:12. Cattle with antibodies to PI3 virus had a mean activity of 78.3 ± 27.7 units, equivalent to a PI3 SNT titer of 1:48. The positive sera to BRS and BVD viruses showed mean activities of 65.1 ± 27.7 units and 64.6 ± 22.3 units, values approximately equivalent to BRS and BVD SNT titers of 1:54 and 1:96, respectively.

Antibodies to IBR and BVD viruses were found in 37% and 40% of the cattle, while PI3 and BRS viral antibodies were found in 93% and 78% of the cattle, respectively. Antibodies to IBR and BVD viruses were found to be less common in the Prince Albert-Tisdale region ($p \leq 0.001$) (Table 2). This was influenced in part by the large number of sera from the Melfort Research Station, where the samples showed only a 5.5% reactor rate for IBR virus and a single trace reaction for antibodies to BVD virus. Nevertheless, the remaining sera from this region still demonstrated low antibody prevalence to IBR ($p \leq 0.001$) and BVD ($p \leq 0.05$) viruses. In contrast, antibodies to IBR ($p \leq 0.001$), BVD ($p \leq 0.001$), and BRS ($p \leq 0.01$) viruses were notably more frequent in the Weyburn-Moosomin-Yorkton region. The prevalence of antibodies to IBR and BVD viruses in cattle from Alberta was also noted to be low ($p \leq 0.001$), though PI3 and BRS viral antibodies followed the usual pattern.

Antibodies were less prevalent in male than in female animals (Table 3), the difference being significant for IBR ($p \leq 0.01$), BRS ($p \leq 0.001$) and BVD ($p \leq 0.01$) viral antibodies. Analysis of age differences (Table 4) showed that the low prevalence in males was confined to young animals between four and ten months of age. Antibodies to IBR, PI3, and BVD viruses became progressively more common in older animals, reactor levels increasing substantially at one year of age. However, only a slight increase was seen with PI3 virus.

Antibodies to IBR and BVD viruses were found to be more common in adult Holstein cattle ($p \leq 0.001$) when compared with adult cattle of other breeds (Table 5). Antibodies to IBR virus were much less frequent in young Herefords ($p \leq 0.001$). A low prevalence of antibodies to IBR and BVD viruses was seen in Charolais cattle ($p \leq 0.001$), but this was heavily influenced by the large number of seronegative Charolais animals from the Melfort Research Station. The remaining animals in this breed had a normal level of reactivity.

A comparison was made of reactor rates in cattle at entry to the feedlots located on the Melfort Research Station, the commercial feedlot, and the ROP and Vermilion bull test stations (Table 6). The locally-bred calves had substantially lower reactor rates to IBR and BVD viruses ($p \leq 0.001$) than introduced calves. In contrast, the prevalence of antibodies to BRS virus was higher in locally-bred calves ($p \leq 0.001$).

Table 3. Prevalence of antibodies to IBR, PI3, BRS, and BVD viruses in sera according to sex (where data available)

Sex	Number of animals	Prevalence of antibodies (%) to: IBR	PI3	BRS	BVD
Male	993	36.4	93.1	69.7	37.4
Female	703	42.3	95.3	90.6	46.9

Reactor rates were found to be considerably higher in animals that had been vaccinated with IBR, BRS, and BVD vaccines prior to entry to the commercial feedlot, research station, and bull test stations (Table 7). Vaccination with PI3 vaccine had little effect on subsequent reactivity.

Discussion

The number of samples tested in the present survey was not large, because sampling was restricted to animals for which data on age, sex, breed, and vaccination history were generally available. The results, however, are considered to be a useful guide to current antibody prevalence in western Canada.

Antibodies to IBR virus were found in 37.8% of cattle tested, and represented at least 59.5% of properties tested. The serum reactor rate is somewhat lower than the level of 48.1% reported previously by Saunders (8) in Saskatchewan cattle in 1972. The difference is misleading, however, because of the different age composition of the sample. The results in the one-to-three-year-old age group and in older animals (48.8% and 81.6%, respectively) were actually somewhat higher than those reported by Saunders (8) for the same age groups (33.8% and 57.6%, respectively). The present results also appear higher than those reported by Niilo *et al* (7) in 1962 and by Darcel (9) in 1973 in equivalent age groups of cattle in Alberta. This suggests that some increase in IBR antibody prevalence may have occurred in recent years, possibly as a result of changed vaccination practices.

The vast majority of the animals (93.9%) possessed antibodies to PI3 virus, representing almost all of the farms that were sampled. This level was much higher than that reported in Alberta by Niilo *et al* (7), who found only 22.7% of cattle to have antibody titers to this virus. However, similar high prevalences of antibodies to PI3 virus have been reported frequently in cattle in the USA (14–17). Although increased use of PI3 vaccine could account for the higher prevalence of antibodies to this virus, antibody titers were also found to be high in unvaccinated animals, suggesting that active infection may be involved.

The prevalence of antibodies to BRS virus was high, involving 78.5% of the animals and 86.6% of properties tested. This result compares closely with a reactor rate of 76% in the small sample of 49 animals from near Saskatoon reported by Moteane *et al* (10) in 1977, and is intermediate between the 35.9% reported by Elazhary (18) in cattle in Quebec and the 95% reported by Lynch and Derbyshire (19) in cattle in Ontario in 1983.

There are no previous data on the prevalence of antibodies to BVD virus in Saskatchewan cattle. The presence of antibody in 40.6% of sera involving at least 66.7% of the properties tested was considerably lower than the 94.9% reported in 1984 by Stone *et al* (11) in cattle in southern Alberta, but appears only slightly lower than values reported in the USA (15,17).

The distribution of antibodies to the four viruses within Saskatchewan varied, antibodies to IBR and BVD viruses being less prevalent in the Prince Albert-Tisdale districts, and more prevalent in the Weyburn-Moosomin-Yorkton districts. Antibodies to BRS virus were also more frequent in the latter area.

Cattle from Alberta had a high pattern of reactivity for PI3 and BRS viruses, a result similar to that seen in Saskatchewan. However, IBR reactivity was low, even lower than that reported in earlier surveys by Niilo *et al* (7) and Darcel (9) in cattle in Alberta. The low prevalence of BVD viral antibodies was in marked contrast with the moderate to high prevalence of antibodies to BVD virus in Alberta cattle reported in 1984 by Stone *et al* (11). The difference may be a result of small sample size, or be a consequence of regional variation, as considerable regional differences were noted by Stone *et al* (11).

The lower prevalence of antibodies to IBR, BRS, and BVD viruses in males compared with females was unexpected. As the lower prevalence in males was confined to the young age group, the difference may be related to management fractors.

Table 4. Prevalence of antibodies to IBR, PI3, BRS, and BVD viruses by age (where known)

Age	Sex	Number of sera	Prevalence of antibodies (%) to: IBR	PI3	BRS	BVD
4–10 months	Male	515	16.3	88.9	48.7	20.0
	Female	184	19.9	94.8	94.0	27.8
1 year	Male	242	55.6	96.6	87.5	47.6
	Female	312	34.0	92.7	83.7	42.7
2 year	Male	224	55.8	99.1	95.1	63.4
	Female	63	54.0	100.0	96.8	52.4
3–6 years	Male	24	62.5	95.8	95.8	75.0
	Female	98	81.6	98.9	97.9	80.6
7–13 years	Male	5	0.0	100.0	100.0	100.0
	Female	28	71.4	100.0	100.0	92.3

Table 5. Prevalence of antibodies to IBR, PI3, BRS, and BVD viruses by breed and age

Breed	Age (years)	Number of sera	Prevalence of antibodies (%) to: IBR	PI3	BRS	BVD
Charolais	<2	404	11.6[a]	92.3	63.4	10.4[a]
	≥2	112	66.9	99.1	98.2	63.4
Hereford	<2	234	20.1	86.3	57.7	33.3
	≥2	109	46.8	100.0	95.4	61.5
Angus	<2	306	42.5	95.8	85.6	44.1
	≥2	24	39.1	95.7	91.3	65.2
Simmental	<2	117	38.1	77.1	55.1	31.4
	≥2	45	60.0	91.1	82.2	17.8
Holstein	<2	1	100.0	100.0	100.0	0.0
	≥2	61	77.0	100.0	98.4	70.5
Other breeds	<2	75	26.5	73.5	56.1	57.1
	≥2	27	51.3	48.7	69.2	79.5
Unknown	<2	114	69.3	94.7	83.3	58.8
	≥2	62	72.6	96.8	95.2	67.7

[a]Antibody prevalence was lowered by the large number of negative sera from Melfort Research Station

Table 6. Prevalence of antibodies to IBR, PI3, BRS, and BVD viruses in locally bred and introduced calves at entry to four feedlots

Farm	Number of calves	Origin of calves	Antibody prevalence (%) to: IBR	PI3	BRS	BVD
Melfort Research Station	253	Local	5.5	98.4	79.4	0.0
Strathmore feedlot	50	Local	3.9	98.0	86.0	16.0
ROP Bull Test Station	267	Introduced	19.8	80.5	31.5	22.5
Lakeland Bull Test Station	80	Introduced	21.5	100.0	60.8	40.5

The prevalence of IBR and BVD viral antibodies was low in animals aged four to ten months, increased considerably at one to two years of age, and increased more gradually thereafter. In contrast, antibodies to PI3 virus were very common in all age groups, only a slight increase being noted at one to two years of age. Antibodies to BRS occupied an intermediate position, being fairly frequent in calves, though still increasing substantially at one year of age. The large numbers of calves with antibodies to PI3 and BRS viruses may have been partially a result of passive transfer of maternal antibody from the high proportion of seropositive dams, though it could also be a consequence of active spread of virus within the calf population.

No major difference between breeds was noted in the prevalence of antibodies to PI3 and BRS viruses. A lower prevalence of IBR antibody was noted in younger animals of the Hereford breed. In contrast, adult Holsteins appeared to have a higher prevalence of IBR and BVD viral antibodies, possibly as a result of more intensive management practices. As indicated in the results, the Charolais cattle held at the Melfort Research Station had only a low prevalence of antibodies to IBR virus and little serological evidence of BVD virus activity, possibly reflecting the closed nature of the herd and lack of previous vaccination.

The prevalence of IBR and BVD viral antibodies was notably lower in calves that were of local origin, in comparison to introduced calves. This possibly reflects the benefits of maintaining a closed herd, as suggested by Martin *et al* (20). However, this observation is difficult to equate with the higher level of BRS viral antibodies found in the closed herds, for which there is no obvious explanation.

As reactivity to PI3 virus was already high, there appeared to be little serological response to vaccination with this virus. In contrast, animals with a history of previous vaccination against IBR, BRS, and BVD viral infections possessed considerably higher prevalences of antibodies to the respective viruses. This finding was less marked with BRS virus, where over half the unvaccinated animals possessed antibodies to the virus.

In conclusion, PI3 virus appeared to be widespread in cattle in Saskatchewan, the prevalence being little

Table 7. Effects of vaccination of calves with IBR, PI3, BRS, and BVD viral vaccines on subsequent serological reactor rates at entry to the feedlots

Virus	Not vaccinated: No. of animals	Not vaccinated: Reactor rate (%)	Vaccinated: No. of animals	Vaccinated: Reactor rate (%)
IBR	493	6.9	169	31.9
PI3	493	91.1	169	92.3
BRS	638	57.4	27	77.7
BVD	559	10.6	104	38.5

influenced by factors such as breed, age, vaccination practices, or sex. Antibodies to BRS virus were also common in all breeds and districts, but appeared to be relatively less frequent in young, male, or unvaccinated animals. In contrast, antibodies to IBR and BVD viruses were much less prevalent in Saskatchewan cattle, especially in the Prince Albert-Tisdale region. Reactor rates for these two viruses were even lower in young, male, and unvaccinated animals. Antibodies to IBR virus were also lower in Herefords, but Holsteins appeared to have a higher prevalence of antibodies to IBR and BVD viruses. Despite vaccination, only about one-third of vaccinated animals had detectable antibodies to IBR and BVD viruses. Although numbers of cattle sampled from Alberta were relatively small, the prevalence of PI3 and BRS virus infections was very similar to that generally seen in cattle in Saskatchewan, though IBR and BVD reactivity was somewhat lower.

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